I. PETITION FOR EXTENSION OF TIME

Pursuant to 37 C.F.R. § 1.136(a), Applicants petition for an extension of time of one-month to and including February 24, 2003, in which to file the instant response. Pursuant to 37 C.F.R. § 1.17, a check in the amount of $55.00 is enclosed, which is the process fee for a one-month extension of time. If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the instant response, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/UTSB:675.

II. AMENDMENT

In the specification:

Please amend the paragraphs beginning on page 11, line 6, and ending on page 13, line 9:

FIG. 1. General scheme of the basis of a preferred embodiment of the invention. A library of proteins expressed in the periplasmic space of bacteria is contacted with a fluorescent reagent. Bacterial clones expressing a protein having a desired activity (*e.g.*, either binding of the probe or enzymatic conversion to a product) become fluorescently labeled. The fluorescent cells can subsequently be isolated by FACS.

FIGs. 2A-2C. Isolation of affinity improved mutants of an anti-digoxin antibody by two rounds of sorting. A library of scFv mutants in which three residues in the light chain had been randomized was constructed as described in Example 2. A total of 2.5×10^6 transformants were grown in liquid media, labeled with 100 nM digoxin-BODIPY™ and fluorescent cells falling within the window shown in the rightmost panel were sorted by FACS. The sorted cells were grown in liquid media, re-labeled and cells falling within the specified window as shown in the center panel were isolated. Following a final round of re-growth the cells were analyzed by FACS (FIG. 2A). Single scFv antibody colonies were picked at random, analyzed and the affinity of the corresponding scFv proteins are reported in Table 1.

FIGs. 3A-3H. Shows strain dependence of periplasmic FACS signal: FIG. 3A TG1/pHEN2.thy; FIG. 3C HB2151/pHEN2.thy; FIG. 3E ABLE™C/pHEN2.thy; FIG. 3G ABLE™K/pHEN2.thy; FIG. 3B TG1/pHEN2.dig; FIG. 3D HB2151/pHEN2.dig; FIG. 3F ABLE™C/pHEN2.dig; FIG. 3H ABLE™K/pHEN2.dig.

FIGs. 4A-4H. Effect of hyperosmotic shock on labeling efficiency: FIG 4A, FIG. 4C, FIG. 4E, FIG. 4G: pHEN2.thy; FIG 4B, FIG. 4D, FIG. 4F, FIG. 4H pHEN2.dig; FIG. 4A and

FIG. 4B, 1xPBS; FIG. 4C and FIG. 4D, 2.5xPBS; FIG. 4E and FIG. 4F 5xPBS; FIG. 4G and FIG. 4H 10xPBS.

FIGs. 5A-5D. Maximizing periplasmic FACS signal in ABLE^{TM}C labeled in 5xPBS using P_{tac} vector and superinfection with M13KO7 (moi of 10) 0.5h pre-induction: FIG. 5A pHEN.thy; FIG. 5C pHEN2.thy/M13K07; pHEN2.dig; FIG. 5D pHEN2.dig/M13K07.

FIGs. 6A-6C. FIG 6A: Phage eluate titers, after each round of panning. FIG. 6B: Polyclonal phage ELISA of purified phage stocks on digoxin-ovalbumin. FIG. 6C: FACScanning naïve library FIG. 6C-1 and rounds one to five (FIG. 6C-2 to FIG. 6C-6) of panning on digoxin-BSA using BODIPY^{TM}-digoxygenin.

FIGs. 7A, 7B. Amino acid and nucleotide sequences of scFv antibody fragments isolated by expression in the periplasm and FACS. FIG. 7A: Heavy chain of dig1 is shown in true font while dig3 is shown in italics underneath. The nucleotide sequences corresponding to the heavy chains of dig1 and dig 3 are given by SEQ ID NO:17 and SEQ ID NO:18, respectively. Dig2 variation from dig 1 is as indicated in underlined text within CDR3. FIG. 7B: Light chain of dig1, 2 and 3 with variations in CDR3 indicated as for heavy chain. The nucleotide sequences corresponding to the light chains of dig1 and dig 3 are given by SEQ ID NO:19 and SEQ ID NO:20, respectively. The underlined four nucleotide variation beginning at nucleotide 99 is given by SEQ ID NO: 21.

FIGs. 8A-8D. Labeling of periplasmic scFv by fluorescently tagged oligonucleotide probe. ABLE^{TM}C cells expressing periplasmic scFv specific for either atrazine as a negative control (FIG. 8A and FIG. 8C) or for digoxin (FIG. 8B and FIG. 8D) were labeled either with: 100nM with digoxigenin-BODIPY™ (FIG. 8A and FIG. 8B) or 100nM of dig-5A-FL (FIG. 8C and FIG. 8D). 10,000 events were recorded using a FACSort flow cytometer at a rate of approximately 1,000 events per second.

FIGs. 9A-9B. Fluorescence discrimination of *E. coli* expressing the enzyme cutinase (an esterase) from control bacteria not expressing the enzyme. *E. coli* DH5a cells were transformed either with the plasmid pBAD18Cm (control cells) or with the derivative plasmid pKG3-53-1 encoding the *Fusarium solani* enzyme cutinase. FIG. 9A. Fluorescence histogram showing the selective labeling of *E. coli* expressing cutinase in the periplasm (pkg3-53-1 containing-cells) using a fluorescent esterase substrate (10μM Fluorescein Dibutyrate) for 30 minutes at 37°C. FIG. 9B. Fluorescence histogram of from selective labeling of cutinase-expressing cells (transcribed from the pKG3-53-4 vector) labeled with a fluorescent pH-Sensitive Dye (1μM LysoSensor Green DND-189) in the presence of cutinase substrate (1mM 4-Nitrophenyl Butyrate). The cells were labeled for 5 minutes at 25°C. Acidification of the periplasm occurred as a result of ester hydrolysis by the cutinase.--

III. REQUEST FOR RECONSIDERATION UNDER 37 C.F.R. §1.111

A. Interview Summary

Applicants' undersigned representative held a teleconference with Examiner Ford on